

retroviral vector MFG. Specifically, a PstI to BamHI fragment from the IRAP cDNA was linked to a synthetic oligonucleotide adapter from the NcoI site (representing the start site of translation for IRAP) to the PstI site (approximately 12 base pairs downstream from the NcoI site) to the MFG backbone digested at NcoI and BamHI in a three part ligation reaction. The straight arrow and the crooked arrow represent unspliced and spliced messenger RNAs respectively. IRAP is encoded by the spliced message. The BAG vector is an MFG derivative. In BAG, the β -gal gene is expressed from a non-spliced LTR-driven message whereas the neo gene is expressed from a SV40 promoter. Both MFG-IRAP and BAG have the psi site required for packaging of the recombinant RNA into virions.

On page 25, paragraph [0098], line 6, delete "SES" and insert therefore --SEQ--. "SES" has been deleted and --SEQ-- has been entered in the following corrected Paragraph [0098]:

[0098] In a further embodiment regarding the IRAP induced systemic treatment of rheumatoid arthritis, the DNA sequence encoding IRAP or a portion thereof is subcloned into a MoMLV retroviral vector prior to systemic delivery to the patient. Specifically, a recombinant MoMLV-IRAP construction that may be utilized in the treatment of SLE is MFG-IRAP (Figure 1), wherein the DNA sequence encoding IRAP or a portion thereof is SEQ ID NO:3 (Figure 2).

On page 35, paragraph [0135], line 28, delete bracket. In the corrected paragraph [0135] below, the bracket has been removed:

[0135] A human monocyte cDNA library, in lambda gt10, was purchased from Clontech (catalog no. HL1036a). The cDNA library was derived from the human monocyte cell line U937, which had been stimulated with 10nM phorbol 12-myristate 13-acetate for 48 hours before mRNA isolation. The library was screened for IRAP cDNA

clones using a 32P-end labelled oligonucleotide encompassing base pairs 309-391 (as indicated by Carter, et al., 1990, Nature 344: 633-638 [see Figure 1 at p. 634]). The probe was antisense. Positive clones were plaque purified from agar plates to nitrocellulose filters. Two positive phage clones with EcoRI flanking sites were isolated and subcloned into the EcoRI site of pUC18 and were shown to have identical restriction maps. One of the clones was then sequenced. The 5' end of the positive cDNA was truncated at bp 93 and replaced with 81 bp of nonsense DNA (most likely a cloning artifact). DNA downstream from about by 110 was also missing. The missing 5' sequences were replaced via two sequential PCR reactions. In the first reaction, the 5' sense primer covered by 43-199. The 3' antisense primer covered by 553-577 and added a HindIII site to the 3' end. The product of this reaction was taken for a second PCR reaction using the same 3' primer and a 5' primer covering by 1-67 which also added a HindIII site to the 5' end. The second PCR product was digested with HindIII, subcloned into pUC 18, and sequenced. The resulting IRAP insert had the following structure (as indicated in SEQ ID NOS:3 and 4, and Figure 2): a 5' HindIII site followed immediately by bp 1 (again, utilizing the numbering of Carter, et al., 1990, Nature 344: 633-638) the entire IRAP coding sequence, 3' flanking DNA from by 543-577, followed by a 3' HindIII site. This insert was subcloned into the HindIII site of pSV2cat. One of ordinary skill in the art will be aware that any of a multitude of vector molecules, especially DNA plasmid vector molecules with a unique HindIII site, can be substituted for pSV2cat at this stage of the construction of MFG-IRAP. This cDNA for human IRAP (SEQ ID NO:3 as subcloned into pSV2cat) was inserted into MFG by first BamHI-linkering the 3' HindIII site downstream of the stop codon of the IRAP gene, followed by digestion with PstI and BamHI. This PstI/BamHI IRAP fragment was ligated to NcoI/BamHI digested MFG, with the addition of a synthetic oligonucleotide adapter

5'-CATGGAATCTGCA-3' [SEQ ID NO:1]

3'-CTTTAG-5' [SEQ ID NO:2];

from the NcoI site (representing the start site of translation for IRAP initiating Met underlined) to the PstI site (approximately 12 base pairs downstream from

the NcoI site) in a three part ligation reaction, resulting in MFG-IRAP (Figure 1). The initiation codon of IRAP is underlined in SEQ ID NO:1. The resulting plasmid, termed MFG-IRAP, contains the entire coding region of IRAP. This three part ligation involving a synthetic oligo and two DNA fragments is well known by those skilled in the art of cloning. The straight arrow and the crooked arrow in Figure 1 represent unspliced and spliced messenger RNAs respectively. IRAP is encoded by the spliced message.

REMARKS

Applicants have requested correction of typographical errors.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-576-0200.

Respectfully submitted,



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